

# **Analytical Method Development and Validation**

## **CBDA Grant Study Goal:**

Develop and validate sample extraction and analysis procedures that give accurate and reproducible results for environmentally relevant concentrations of pyrethroid insecticides (home and commercial use)

# Analytical Method Development and Validation

## Methods

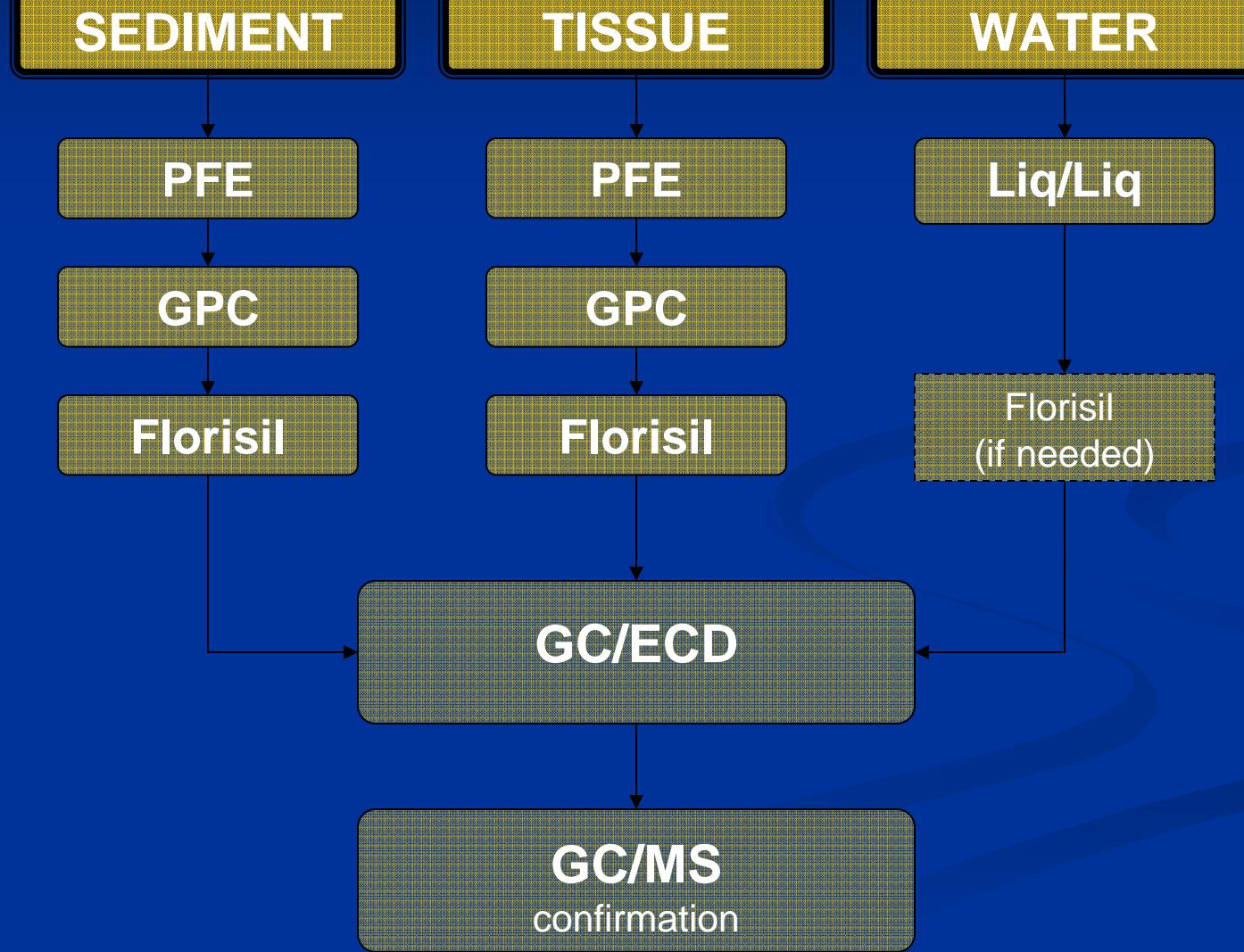
- Compatible with GC and HPLC analyses
- Applicable to water, colloids, sediment and biota
- Applicable at environmentally relevant concentrations
- Amenable to automation for high sample throughput
- Cost effective

# **Analytical Method Development and Validation**

## **Development of Analytical Methods**

- Optimization (sensitivity and selectivity) of instrument methods used for analysis and confirmation (GC-ECD, GC-MS, LC-MS, GC-MS/MS and LC-MS/MS)
- Isomer, enantiomer and stereoisomer identification and stability (deltamethrin, tralomethrin)
- Evaluation of water extraction methods (liq-liq, SPE, SPME)
- Evaluation of sediment and tissue extraction and matrix separation methods (PFE, GPC, Florisil)

# Methods for Pyrethroid Analysis



# **PYRETHROID EXTRACTION AND ANALYSIS METHOD**

**Matrices: Sediment, Tissue and Water**

**Analytes: Bifenthrin, Cyfluthrin, Cypermethrin, Es-fenvaleate,  
Fenpropathrin, Lambda-cyhalothrin and Permethrin**

## **Method Summary**

**Sediment and Tissue: ASE - GPC – Florisil**

**Water: Liquid/liquid or Solid Phase Extraction  
– Florisil (*if needed*)**

**Analysis: High resolution GC/ECD with dual columns**

**Standards obtained from ChemService**

**Second source obtained from AccuStd**

**Reporting Limits: 0.002-0.012 µg/L (water)**

**1-10 ng/g dry wt (sediment)**

**2-6 ng/g fresh wt (tissue)**

**Confirmation by GC/MS**

# Water Sample Extraction

## Liquid-liquid Extraction

Sample Prep: 1 liter unfiltered water sample  
2 liter separatory funnel  
Surrogate/Spike solution

Solvent: Methylene chloride (DCM), 120 mL (2x)

Concentrate and solvent exchange:  
Evaporate using K-D and µK-D (75-80°C),  
Exchange solvent with petroleum ether  
(~ 50 mL, 3x)

Reconstitute: 2 mL isoctane

Analysis: GC/ECD

# PYRETHROIDS IN AMERICAN RIVER WATER

Method Validation: Spike Level 2-10 ng/L

Pyrethroids by GC/ECD	Average	Average	Standard Deviation	MDL=t*Stdev	RL
	% Recovery	Amount Recovered	Amount Recovered	(ng/L)	(ng/L)
Bifenthrin	88.1	1.76	0.31	0.98	2.00
Cyfluthrin	103	4.13	2.19	6.88	12.0
Cypermethrin	101	4.04	1.40	4.39	8.00
Deltamethrin	106	2.12	0.51	1.61	4.00
Es/Fenvalerate	101	2.02	0.55	1.73	4.00
Lambda-cyhalothrin	96.2	1.93	0.51	1.61	4.00
Permethrin	102	6.12	0.69	2.17	4.00

# Sediment/Tissue Sample Extraction

## Pressurized Fluid Extraction

Instrument: Automated Dionex ASE 200  
ASE Conditions

Solvent: Acetone/Dichloromethane (DCM) 50/50  
Program: 100 °C, 1500 psi

Sample Prep: 10 g homogenized sediment/tissue  
7 g Hydromatrix®  
33 mL pre-rinsed extractor cell  
Surrogate/spike solution

Extract Collection: Two 60 mL VOA vials per sample  
Evaporate and solvent exchange: K-D and µK-D (75-80 °C),  
Exchange solvent with petroleum ether (~ 50 mL, 3x)

# Gel Permeation Chromatography

Instrument: J2 Scientific AccuPrep 170, Autoinject 110

## GPC Conditions

Solvent: Dichloromethane (DCM)

Column: 60 x 2.5 cm

Column Packing: 65 g BioBeads, 200-400 mesh

Flow: 5 mL/min

Program: Dump-19 min, Collect-24 min, Rinse-10 min

Sample size: 10 mL DCM extract, 5 mL sample loop

Evaporate and solvent exchange: K-D and  $\mu$ K-D ( $75^{\circ}\text{C}$ ),  
Exchange solvent with petroleum ether (~ 50 mL, 3 times)

# Florisil Column Fractionation

Column Prep: 25 g Preactivated Florisil (130°C, 24 hours) embedded between sodium sulfate with glass wool plug, 11 mm x 300 mm glass column

Reagents:

0 % ethyl ether in petroleum ether (Fraction 1)

6 % ethyl ether in petroleum ether (Fraction 2)

15 % ethyl ether in petroleum ether (Fraction 3)

# Distribution of Pyrethroids Among Florisil Fractions

(0%) Fraction 1

Do Not Collect  
(PCBs and other  
pesticides)

(6%) Fraction 2

Bifenthrin  
Permethrin

(15%) Fraction 3

Cyfluthrin  
Cypermethrin  
Es-fenvalerate  
Lambda-cyhalothrin  
other pyrethroids

# PYRETHROIDS IN AMERICAN RIVER SEDIMENT

Method Validation: Spike Level 5-20 ng/g

Pyrethroids by GC/ECD	Average % Recovery	Average Amount Recovered	STDEV (n=8) Amount Recovered	MDL=t*Stdev (ng/g)	RL (ng/g)
Bifenthrin	106	5.30	0.14	0.43	1.00
Cyfluthrin	108	10.4	0.85	2.55	4.00
Cypermethrin	108	11.1	0.81	2.44	4.00
Deltamethrin	62.0	3.10	0.69	2.08	4.00
Es/Fenvalerate	107	5.39	0.27	0.80	4.00
Lambda-cyhalothrin	104	5.17	0.38	1.15	4.00
Permethrin	99	16.2	1.27	3.80	8.00

# Round Robin Sediment Results

## SAMPLE 1 RESULTS IN TRIPPLICATE

Sample Identification	GC/ECD			Average	STDev
	SED PY 005 dry wt ppb (ng/g)	SED PY 005 dry wt ppb (ng/g)	SED PY 005 dry wt ppb (ng/g)	SED PY 005	SED PY 005
<b>Pyrethroids by GC/ECD</b>					
Bifenthrin	11.9	12.3	15.8	13.3	2.19
Cyfluthrin	2.54	1.53	2.76	2.27	0.66
Cypermethrin	3.23	1.63	3.95	2.93	1.19
Delta/Tralo-methrin	ND	ND	ND	ND	NA
Esfen/Fen-valerate	12.2	11.8	12.0	12.0	0.18
<b>Fenpropathrin*</b>	3.75	4.55	4.29	4.20	0.41
Lambda-Cyhalothrin	3.80	3.74	3.63	3.72	0.08
Permethrin	34.2	36.2	38.6	36.3	2.24
<b>Surrogate (% Recovery)</b>					
Dibromoocatafluorobiphenyl	79.2	78.6	106	88.1	NA
Dibromochlorendated	82.6	78.4	79.6	80.2	NA

\*Estimated MDL

# Round Robin Sediment Results

## SAMPLE 2 RESULTS IN TRIPPLICATE

Sample Identification	GC/ECD			Average	STDev
	SED PY 006	SED PY 006	SED PY 006	SED PY 006	SED PY 006
Pyrethroids by GC/ECD	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	dry wt ppb (ng/g)	
Bifenthrin	15.0	13.3	14.0	14.1	0.86
Cyfluthrin	1.48	1.25	0.66	1.13	0.42
Cypermethrin	1.04	1.56	2.86	1.82	0.94
Delta/Tralo-methrin	ND	ND	ND	ND	NA
Esfen/Fen-valerate	11.2	11.7	12.3	11.8	0.55
<b>Fenpropathrin*</b>	6.62	6.03	6.56	6.40	0.33
Lambda-Cyhalothrin	4.01	3.48	4.51	4.00	0.52
Permethrin	36.7	37.9	42.6	39.1	3.13
<b>Surrogate (% Recovery)</b>					
Dibromoocetafluorobiphenyl	77.4	89.6	94.0	87.0	NA
Dibromochlorendated	81.6	83.6	82.2	82.5	NA

\*Estimated MDL

# Round Robin Sediment Results

## QC RESULTS

Sample Identification	Method Blank  dry wt ppb (ng/g)	American River Sediment LCS	American River Sediment LCSD	SED PY 005 MS
		% Recovery	% Recovery	% Recovery
Pyrethroids by GC/ECD				
Bifenthrin	ND	102	87.8	81.7
Cyfluthrin	ND	75.7	70.2	96.6
Cypermethrin	ND	80.6	71.2	101
Delta/Tralo-methrin	ND	35.7	ND	77.6
Esfen/Fen-valerate	ND	73.1	66.3	112
Fenpropathrin*	ND	76.8	83.7	92.0
Lambda-Cyhalothrin	ND	79.6	70.3	105
Permethrin	ND	95.3	104	104
Surrogate (% Recovery)				
Dibromooctafluorobiphenyl	89.4	88.0	81.2	109
Dibromochlorendated	71.0	72.4	72.8	84.0

\*Estimated MDL

# Round Robin Sediment Results

## GC/ECD vs GC/MS/MS (TSQ)

Sample Identification  Pyrethroid Pesticides	SED PY 005 dry wt. ppb (ng/g)		SED PY 005 dry wt. ppb (ng/g)		SED PY 005 dry wt. ppb (ng/g)	
	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)
Bifenthrin	11.9	10.2	12.3	13.7	15.8	17.4
Cyfluthrin	2.54	1.53	1.53	2.10	2.76	1.91
Cypermethrin	3.23	2.56	1.63	3.07	3.95	6.91
Delta/Tralo-methrin	ND	ND	ND	ND	ND	ND
Esfen/Fen-valerate	12.2	14.4	11.8	16.0	12.0	17.4
Fenpropathrin*	3.75	8.05	4.55	10.40	4.29	9.69
Lambda-Cyhalothrin	3.80	3.20	3.74	4.17	3.63	4.86
Permethrin	34.2	43.2	36.2	51.1	38.6	67.6

\*Estimated MDL

# Round Robin Sediment Results

GC/ECD vs GC/MS/MS (TSQ)

Sample Identification  Pyrethroid Pesticides	SED PY 006 dry wt. ppb (ng/g)		SED PY 006 dry wt. ppb (ng/g)		SED PY 006 dry wt. ppb (ng/g)	
	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)	GC/ECD	GC/MS/MS (TSQ)
Bifenthrin	15.0	18.7	13.3	17.0	14.0	19.0
Cyfluthrin	1.48	3.14	1.25	1.09	0.66	1.96
Cypermethrin	1.04	2.83	1.56	2.92	2.86	3.51
Delta/Tralo-methrin	ND	ND	ND	ND	ND	ND
Esfen/Fen-valerate	11.2	16.1	11.7	15.6	12.3	18.1
Fenpropathrin*	6.62	8.20	6.03	8.28	6.56	12.2
Lambda-Cyhalothrin	4.01	4.18	3.48	4.10	4.51	4.64
Permethrin	36.7	73.4	37.9	68.7	42.6	83.0

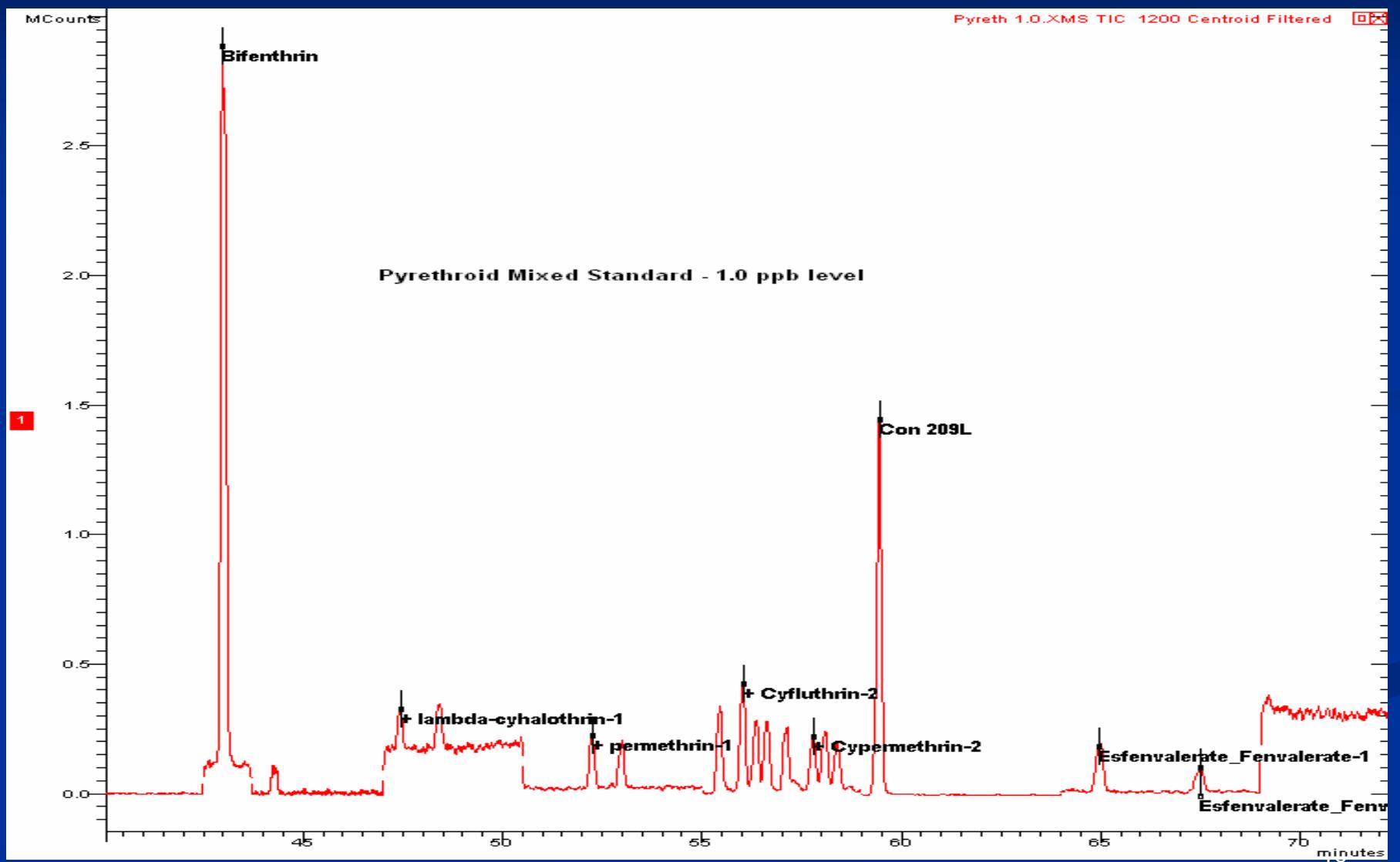
\*Estimated MDL

# Retention times, Q1-Q3 Transitions and Collision Energy

<u>RT</u>	<u>Analyte</u>	<u>Q1</u>	<u>Q3</u>	<u>Collision Energy (-volts)</u>
43.916	Bifenthrin	181	166	15
48.294	Lambda-cyhalothrin-1	181	152	25
49.296	Lambda-cyhalothrin-2	181	152	25
53.156	Permethrin-1	183	153+163	10
53.909	Permethrin-2	183	153+163	10
56.384	Cyfluthrin-1	163	127	10
57.021	Cyfluthrin-2	163	127	10
57.327	Cyfluthrin-3	163	127	10
57.622	Cyfluthrin-4	163	127	10
58.164	Cypermethrin-1	163	127	10
58.876	Cypermethrin-2	163	127	10
59.191	Cypermethrin-3	163	127	10
59.510	Cypermethrin-4	163	127	10
64.970	Es/fenvalerate	167	125	15
66.453	Es/fenvalerate	167	125	15
71.039	Deltamethrin	181	152	20

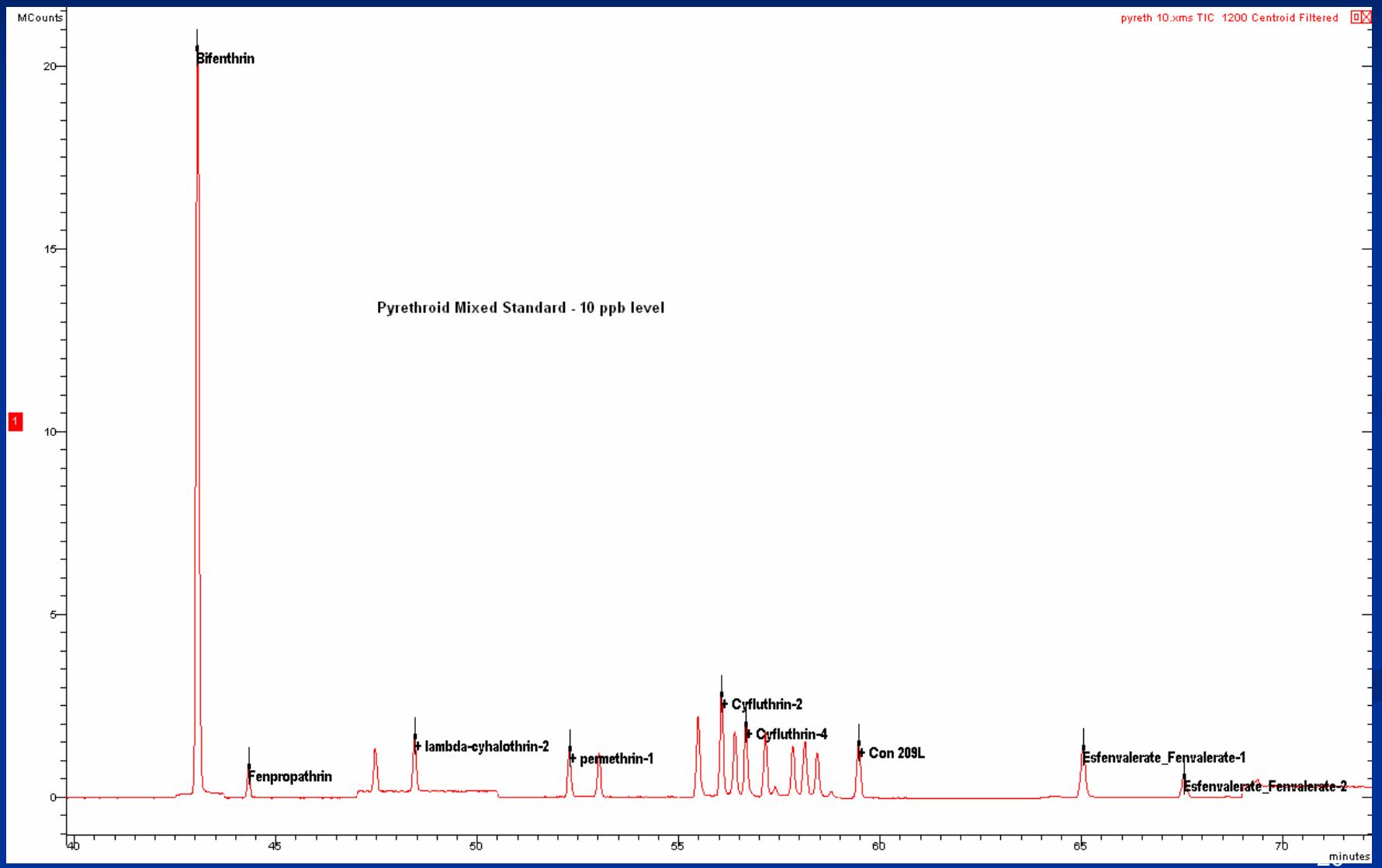
# Pyrethroid Mixed Standard MS/MS

1.0 ppb

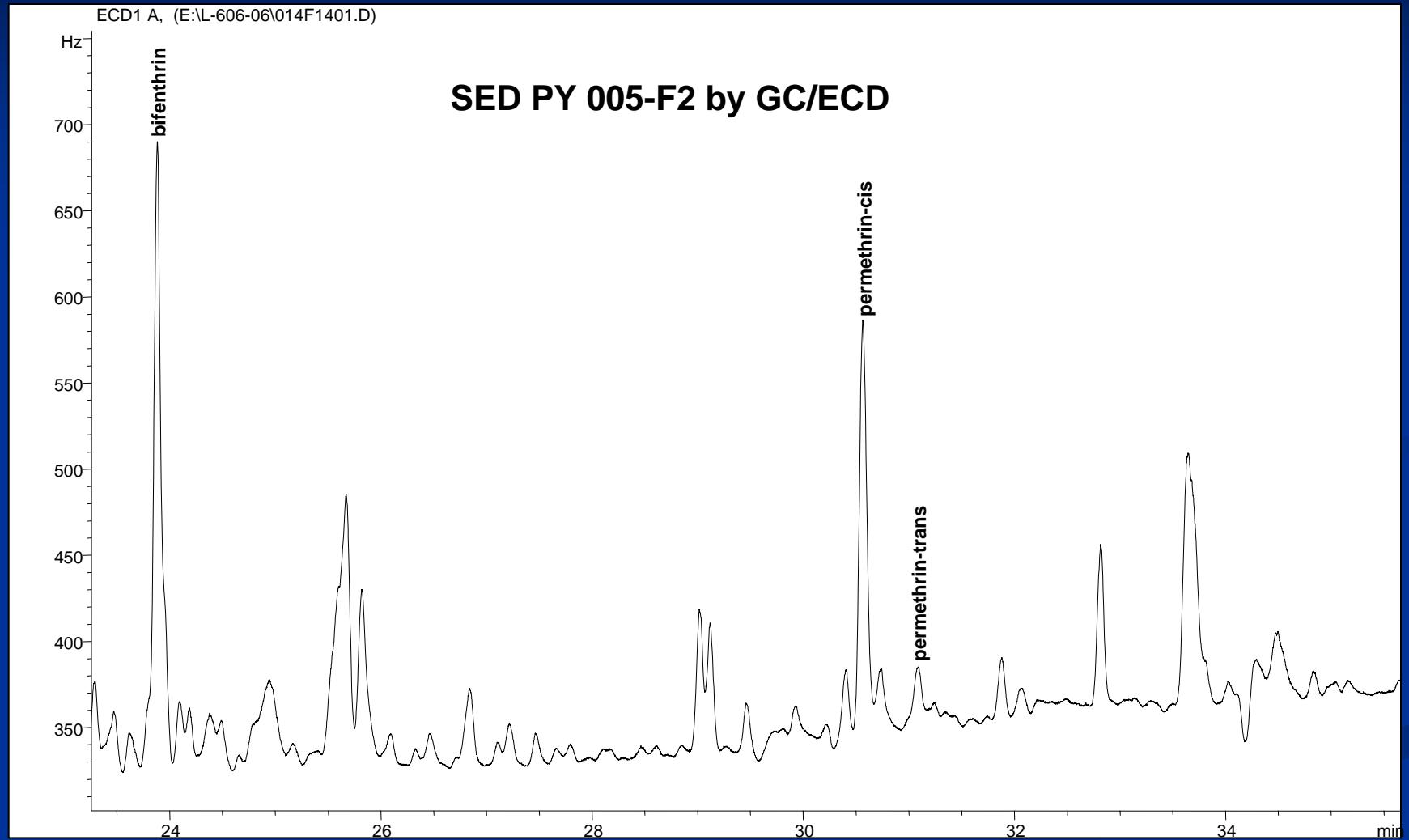


# Pyrethroid Mixed Standard

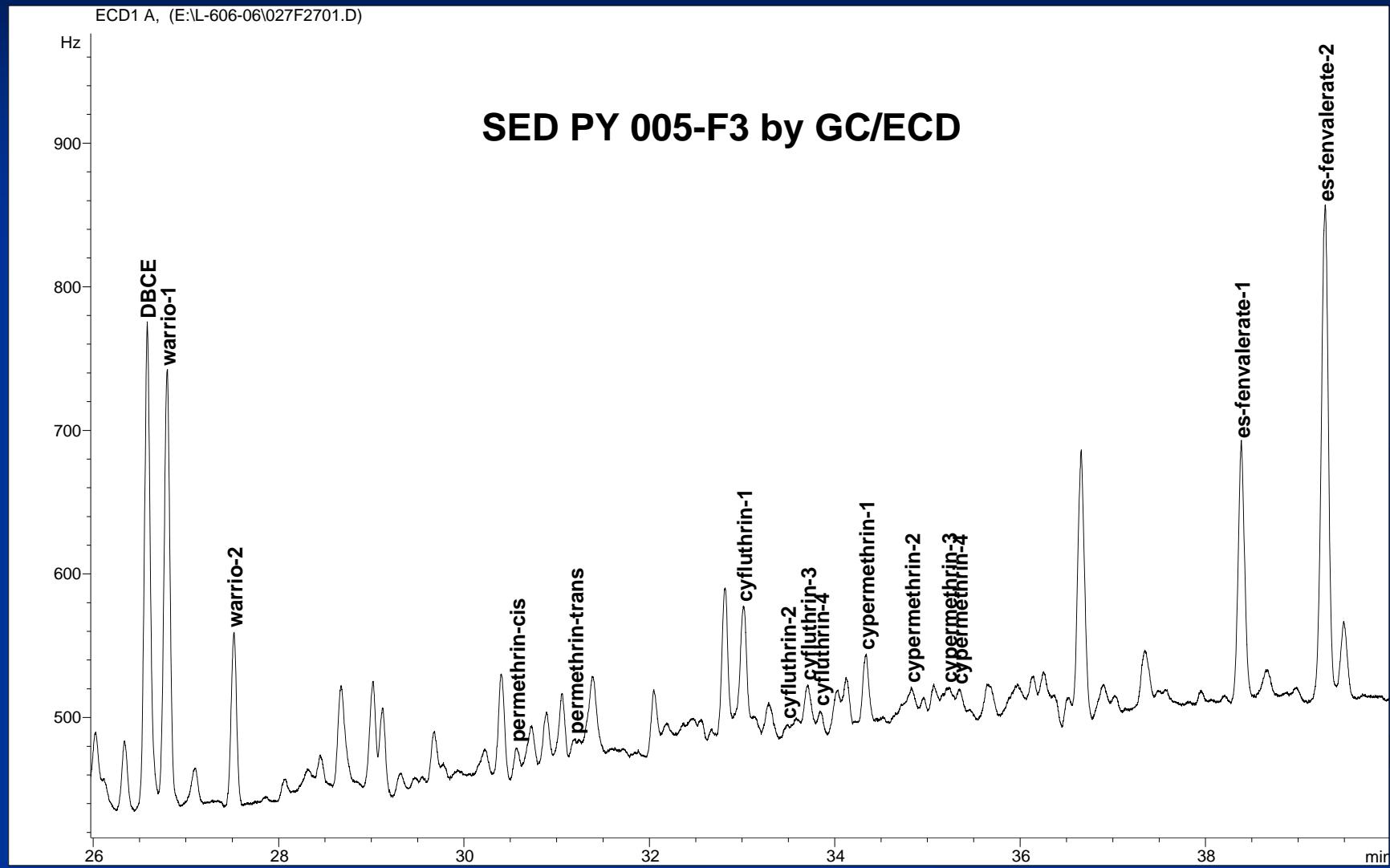
## 10 ppb



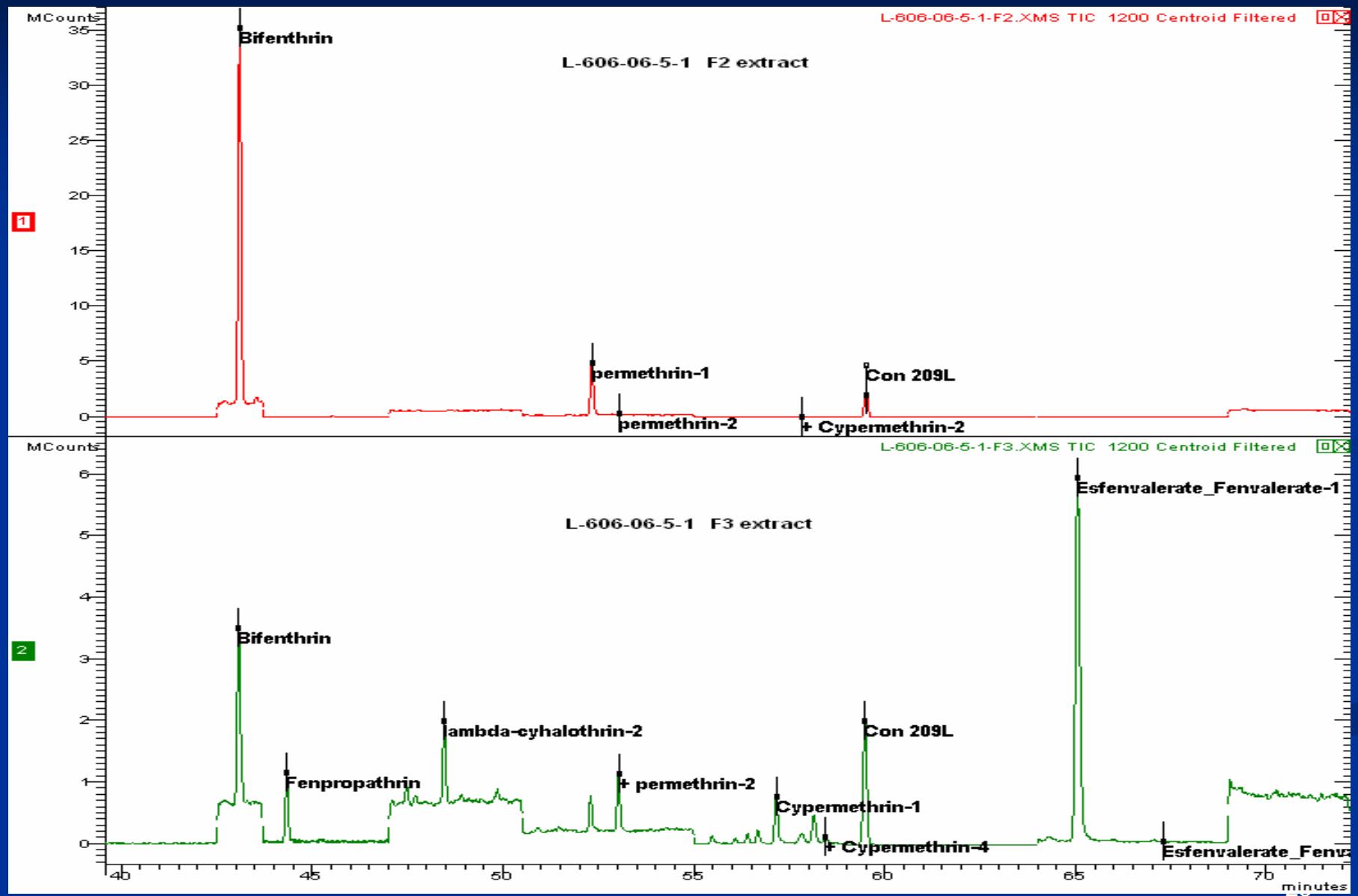
# Round Robin Sediment Extract



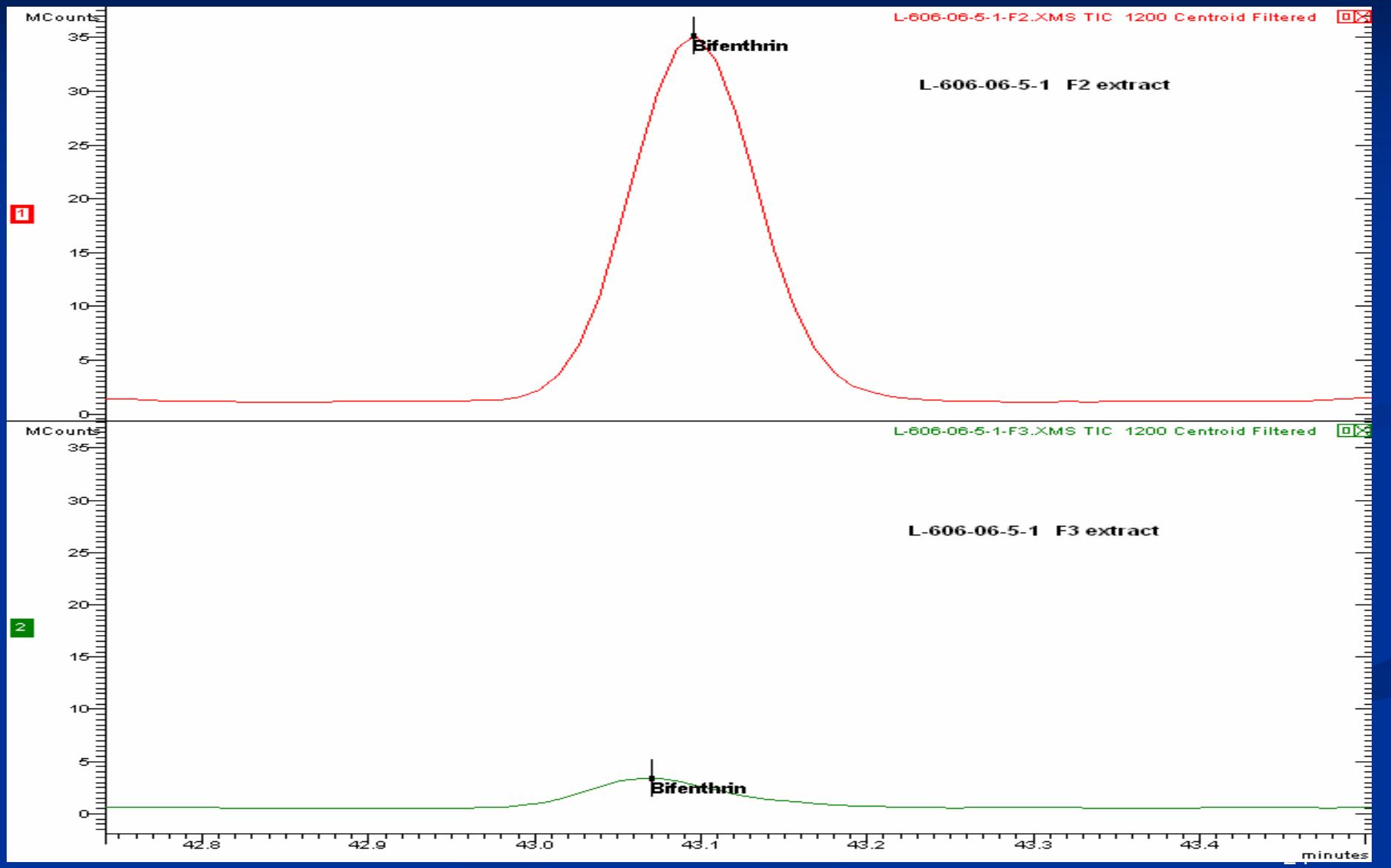
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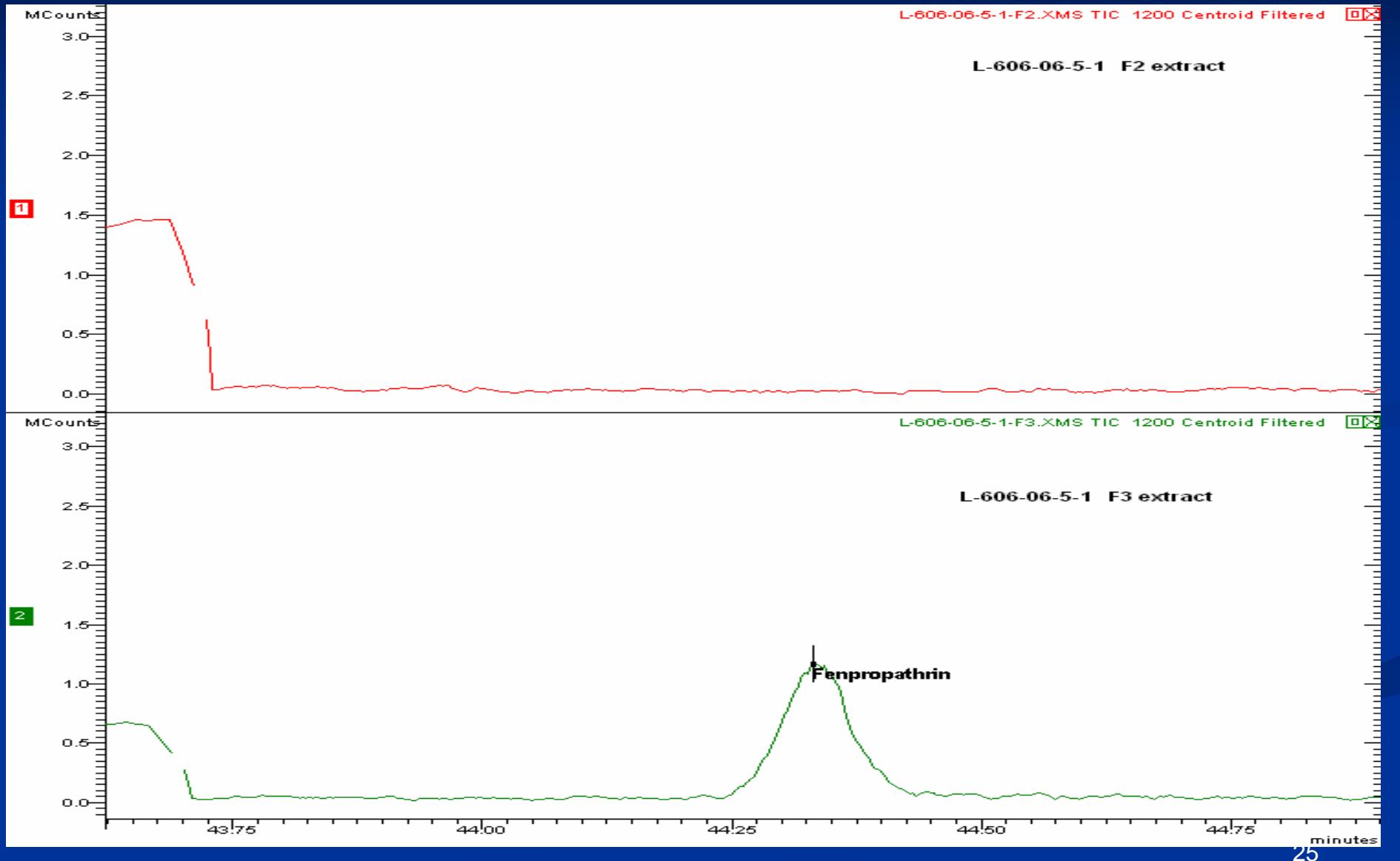
# Round Robin Sediment Extract



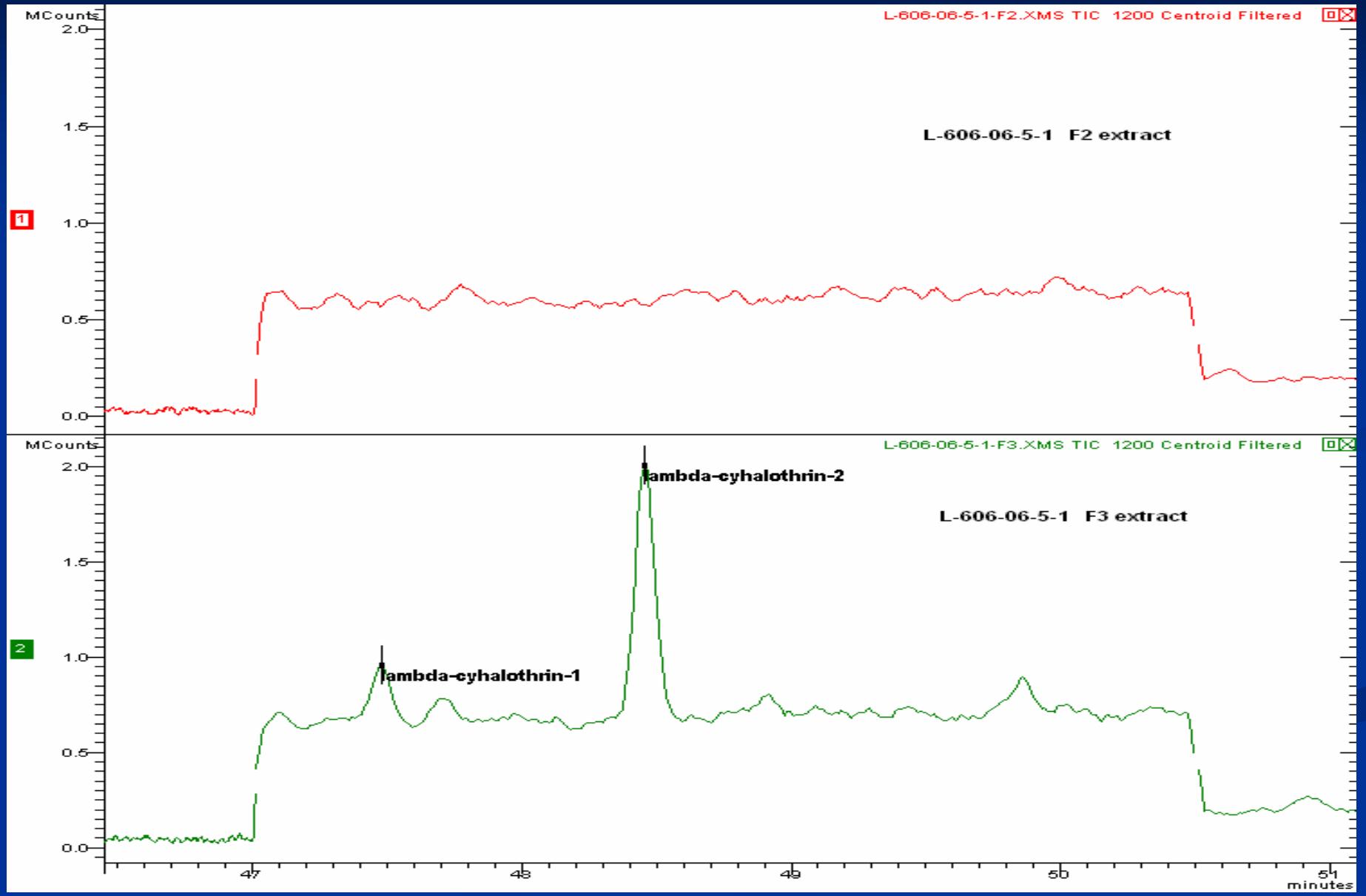
# Round Robin Sediment Extract



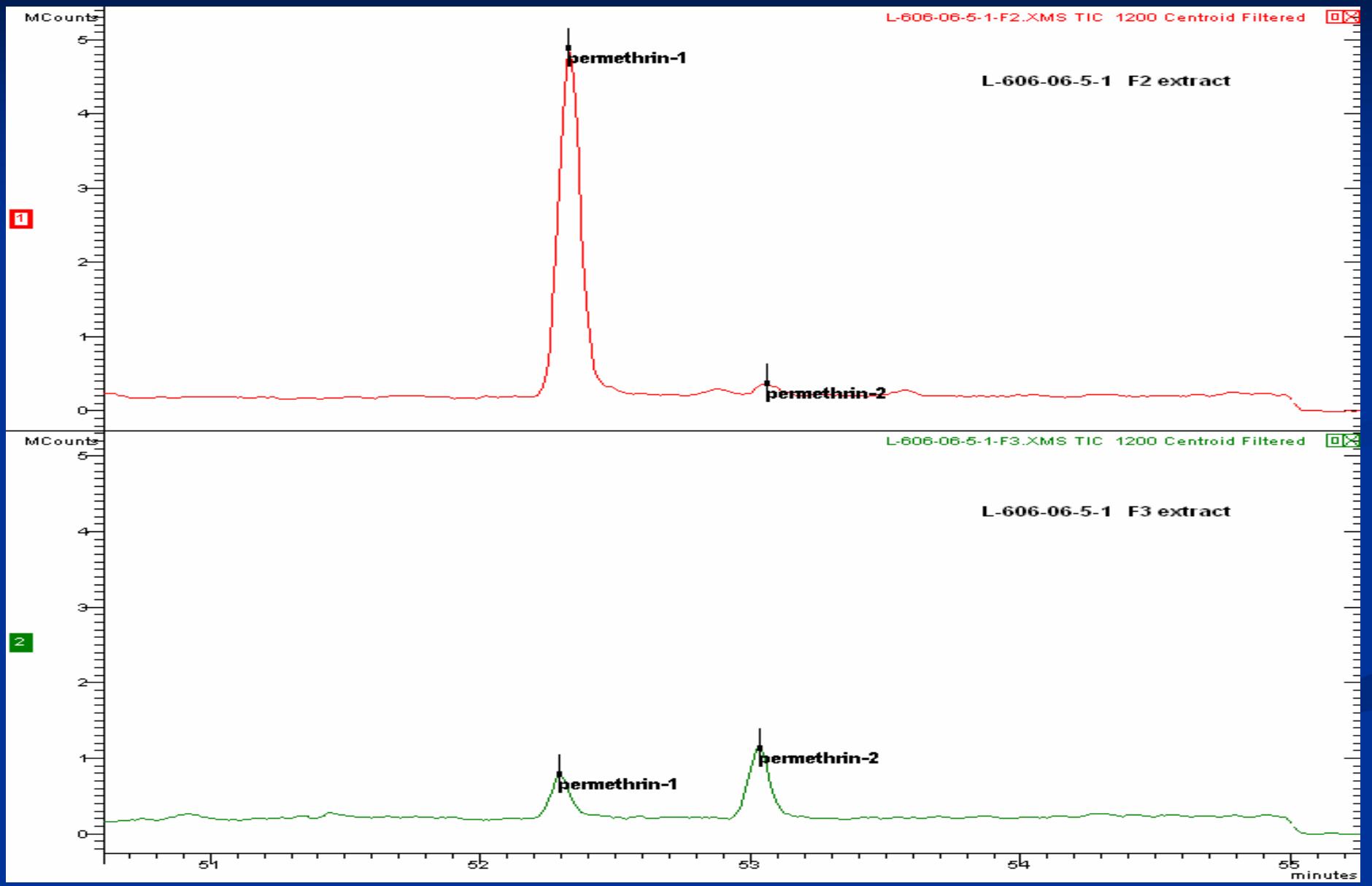
# Round Robin Sediment Extract



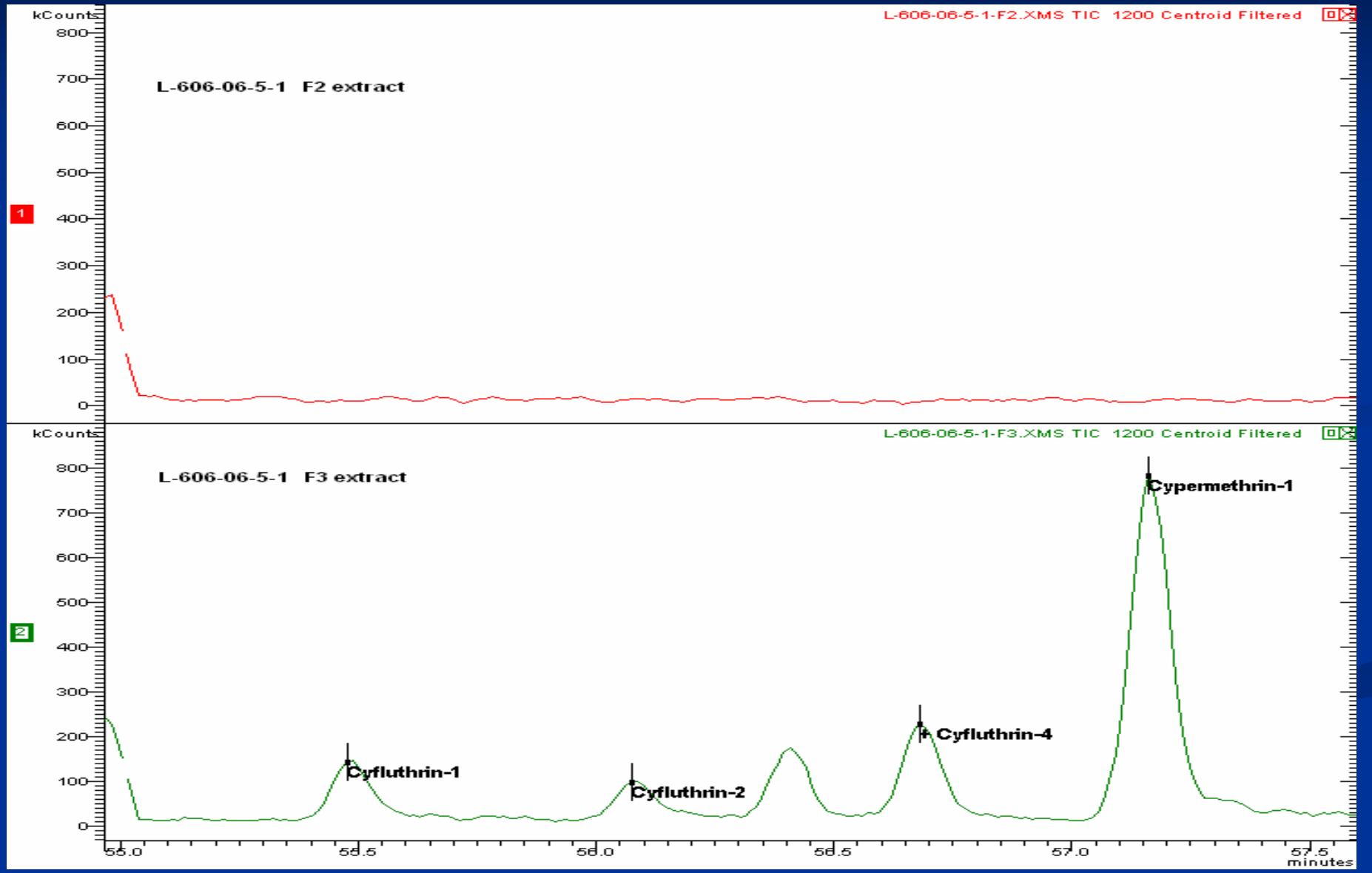
# Round Robin Sediment Extract



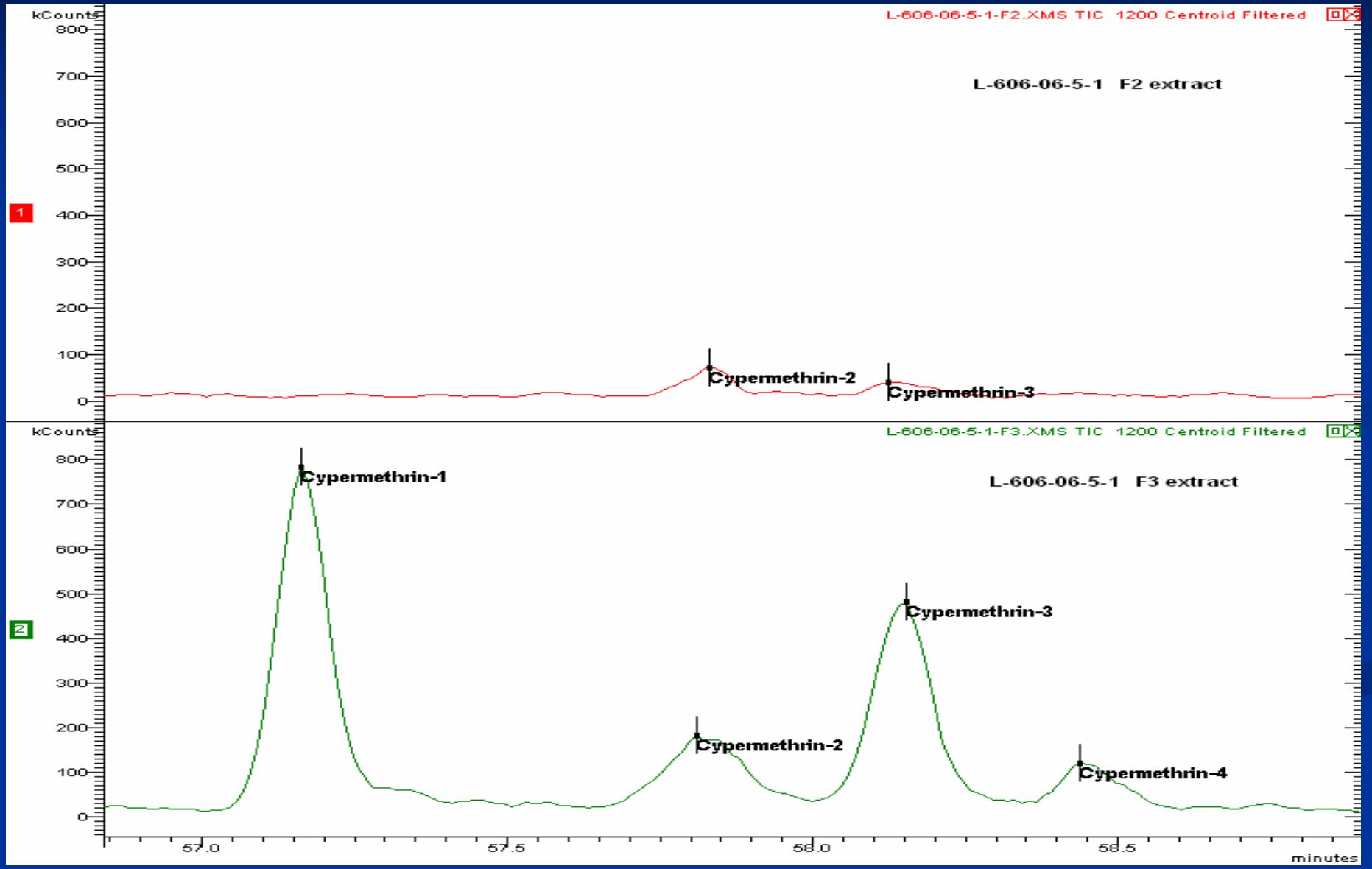
# Round Robin Sediment Extract



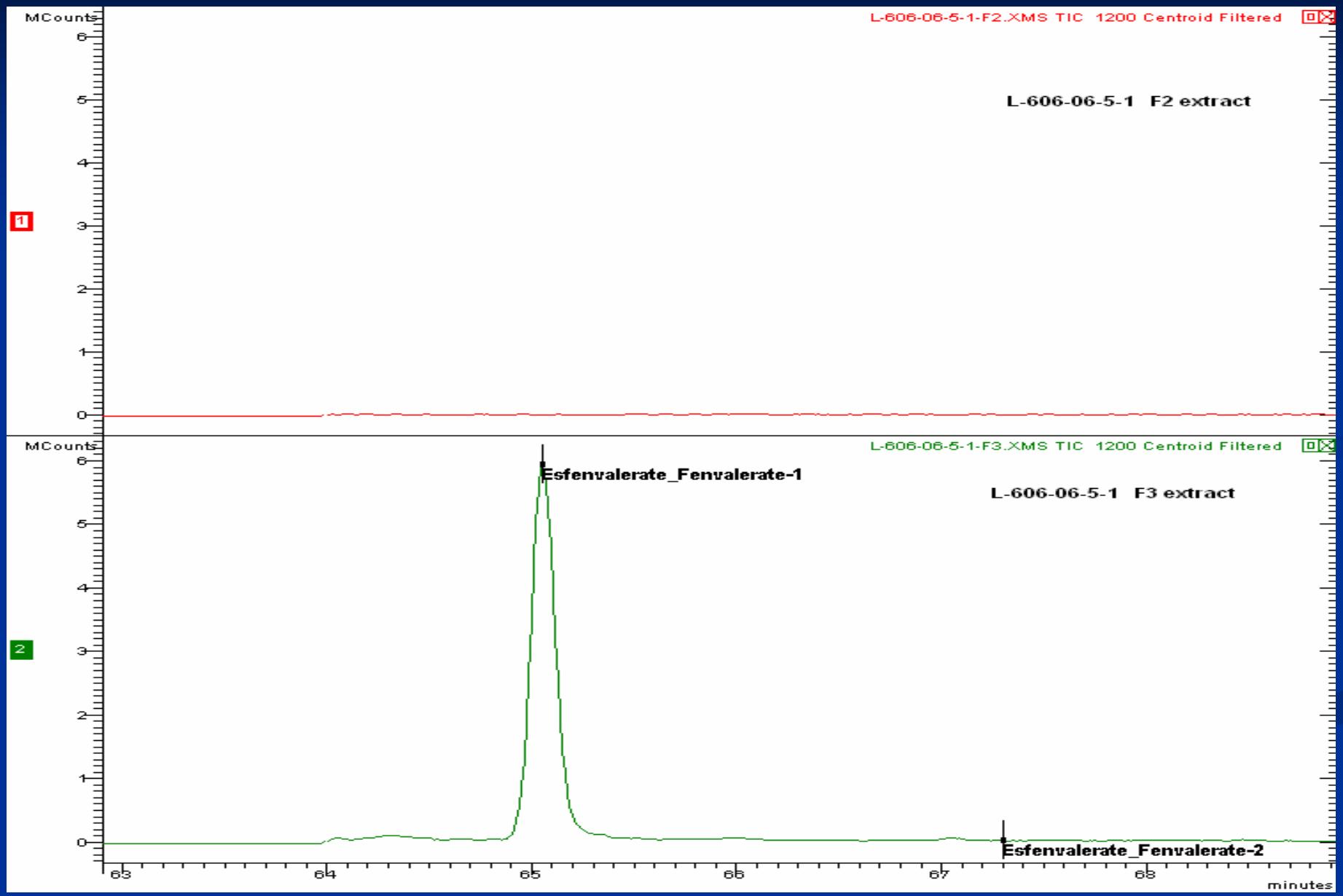
# Round Robin Sediment Extract



# Round Robin Sediment Extract



# Round Robin Sediment Extract



# PYRETHROID TISSUE VALIDATION

## American River Hatchery Trout, Spike Level 4-16 ng/g

Pyrethroid Pesticides	AVERAGE % Recovery	AVERAGE Amount Recovered	STD DEV (n=7) Amount Recovered	MDL=t*Stdev Fresh Wt. ppb (ng/g)	RL Fresh Wt. ppb (ng/g)
Bifenthrin	88.1	3.52	0.21	0.65	2.00
Cyfluthrin	98.7	15.8	0.86	2.72	6.00
Cyhalothrin-Lambda	89.1	3.56	0.47	1.49	4.00
Cypermethrin	74.3	5.94	0.53	1.660	4.00
Deltamethrin	41.8	1.67	0.19	0.60	2.00
Esfenvalerate	52.8	2.11	0.53	1.68	4.00
Fenpropathrin*	85.0	3.40	0.43	1.34	4.00
Permethrin	97.2	11.7	0.71	2.23	6.00

\*Estimated MDL

# PYRETHROID METHOD DETECTION LIMITS AND REPORTING LIMITS FOR WATER, SEDIMENT AND TISSUE

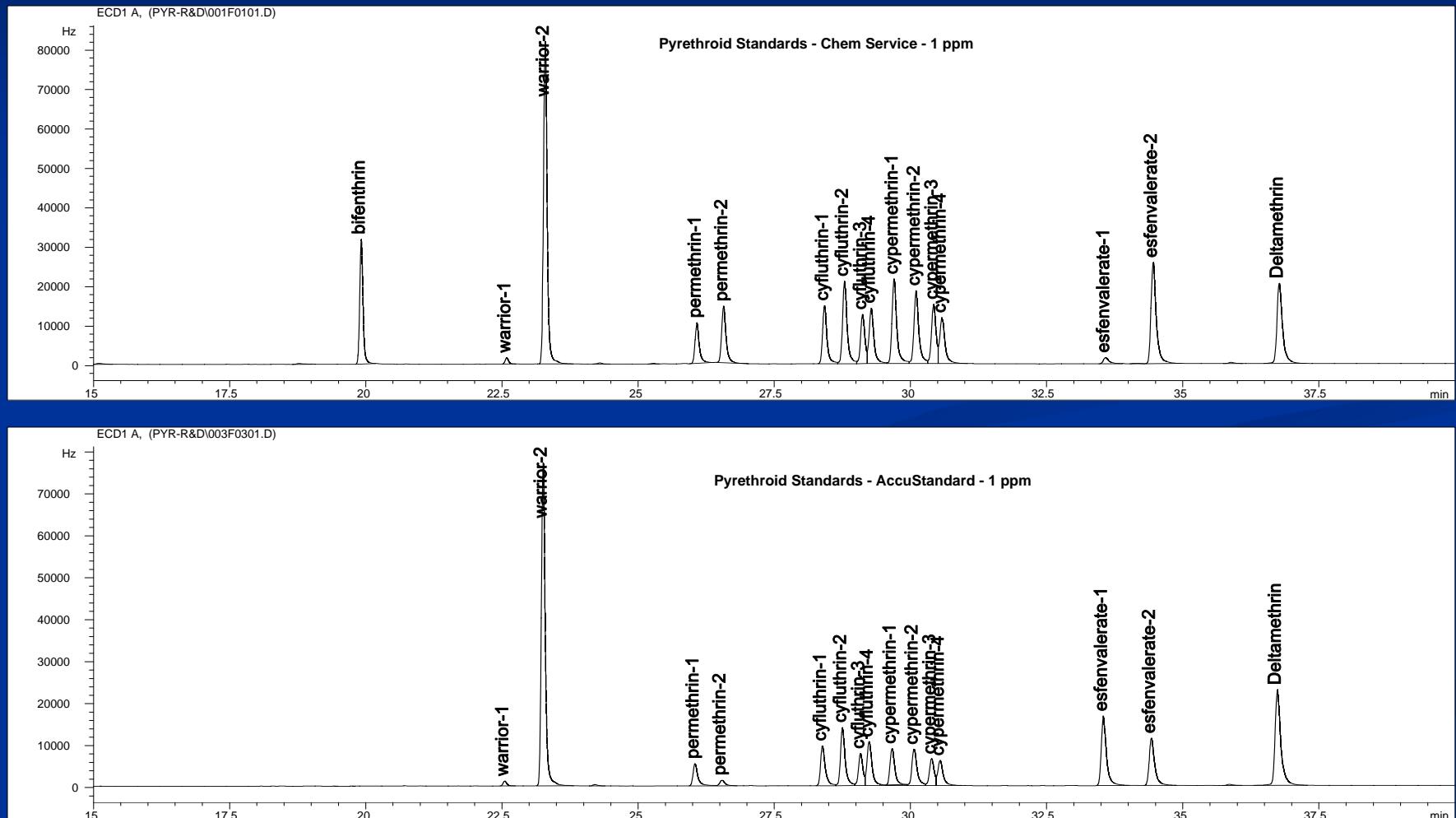
## By GC/ECD

Sample Matrix	Water		Sediment		Tissue	
	MDL ppb (ug/L)	RL ppb (ug/L)	MDL Dry wt ppb (ng/g)	RL Dry wt ppb (ng/g)	MDL Fresh wt ppb (ng/g)	RL Fresh wt ppb (ng/g)
<b>Pyrethroid Pesticides</b>						
Bifenthrin	0.001	0.002	0.50	1.00	0.65	2.00
Cyfluthrin	0.006	0.012	2.00	4.00	2.70	6.00
Cypermethrin	0.004	0.008	2.00	4.00	1.70	4.00
Deltamethrin	0.002	0.004	2.00	4.00	0.60	2.00
Es/Fenvalerate	0.002	0.004	2.00	4.00	1.70	4.00
Fenpropathrin*	0.002	0.004	2.00	4.00	1.35	4.00
Lambda-cyhalothrin	0.002	0.004	2.00	4.00	1.50	4.00
Permethrin	0.002	0.004	4.00	8.00	2.20	6.00

\*Estimated MDL

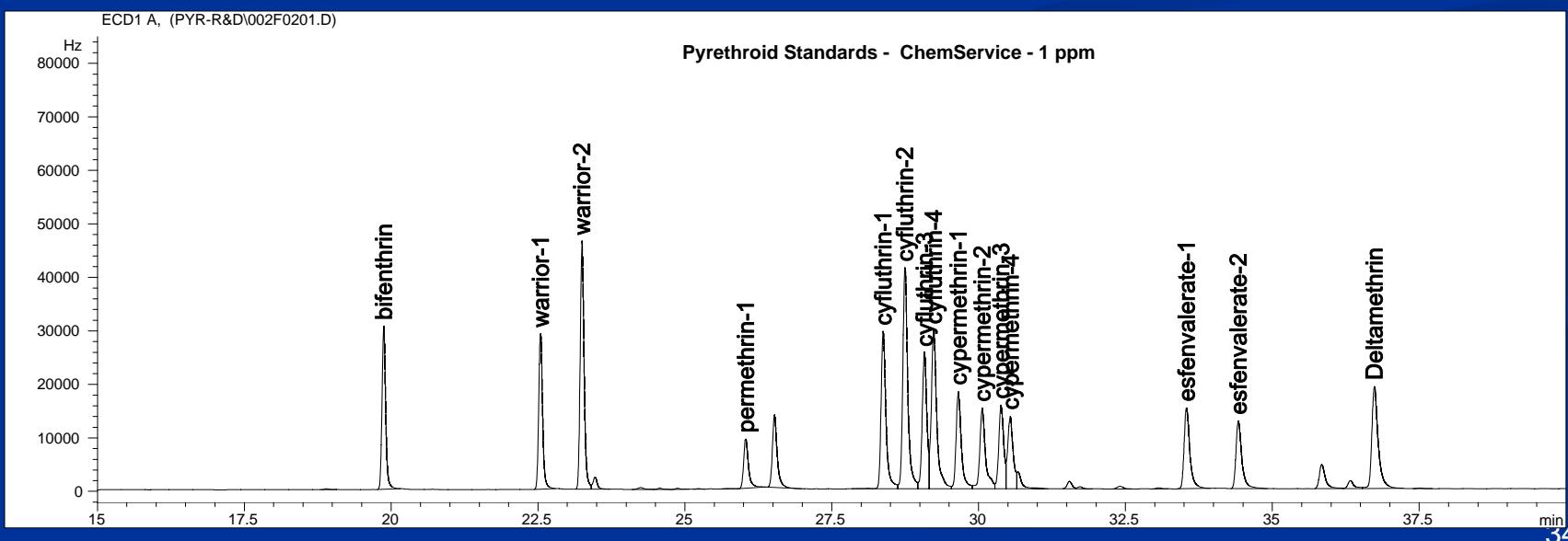
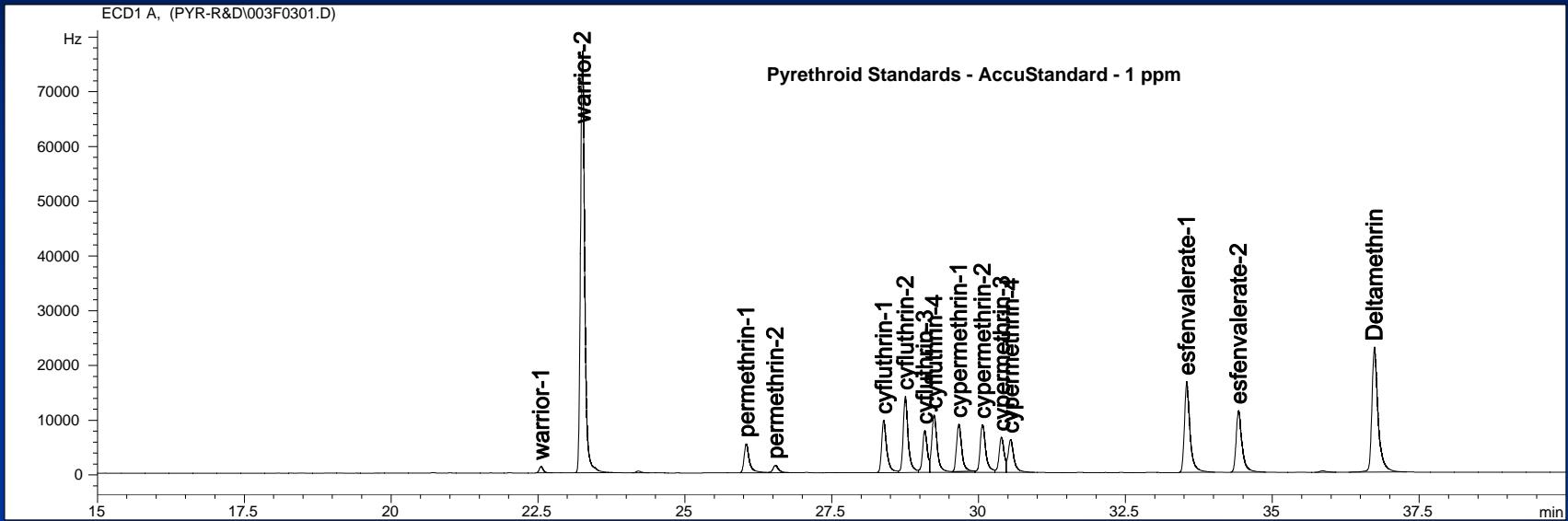
# PYRETHROID STANDARD MATERIAL

## DIFFERENCE IN ISOMER RATIOS BY VENDOR



# PYRETHROID STANDARD MATERIAL

## DIFFERENCE IN ISOMER RATIOS BY VENDOR



# ADDITIONAL PYRETHROID ANALYTES

## Fortified American River Water

Spike Level: 20 ng/L

Extraction Method Pyrethroids by GC/ECD	AR 1 liq/liq	AR 2 liq/liq	AR 3 liq/liq		
	% Recovery	% Recovery	% Recovery	AVERAGE % Recovery	STD DEV
Allethrin	109	98.4	108	105	5.85
Flucythrinate	88.3	83.3	84.3	85.3	2.65
Phenothrin	87.5	88.5	95.5	90.5	4.36
Prallethrin	94.7	86.8	92.6	91.4	4.09
Tetramethrin	112	114	116	114	2.00
Tralomethrin	43.5	51.3	56.2	50.3	6.40
Surrogate (%Rec)					
DBOB	90.0	98.3	112	100	11.1

# ADDITIONAL PYRETHROID ANALYTES

## Fortified American River Water

Spike Level: 40 ng/L

Extraction Method	AR 1				
	SPE-Acidified	AR 2	AR 3	AVERAGE % Recovery	STD DEV
Pyrethrins by GC/ECD	% Recovery	% Recovery	% Recovery		
Allelathrin	113	101	105	106	6.11
Flucythrinate	92.5	91.8	88.8	91.0	1.97
Phenothrin	97.2	94.0	97.3	96.2	1.88
Prallelathrin	118	107	109	111	5.86
Tetramethrin	128	113	119	120	7.55
Tralomethrin	85.6	80.2	77.0	80.9	4.35
Surrogate					
DBOB	95.2	86.1	85.3	88.9	5.50

# ADDITIONAL PYRETHRROID ANALYTES

## Fortified American River Sediment

Spike Level: 4 ng/g

Extraction Method	AR 1	AR 2	AR 3	AVERAGE % Recovery	STD DEV
	ASE	ASE	ASE		
Allelthrin	126	101	110	112	12.7
Flucythrinate	113	105	108	109	4.04
Phenothrin	101	99.2	77.7	92.6	13.0
Prallelthrin	122	103	103	109	11.0
Tetramethrin	120	106	127	118	10.7
Tralomethrin	93.0	91.6	94.5	93.0	1.45
Surrogate (%Rec)					
DBOB	126	104	100	110	14.0

# SUMMARY

- **Methods developed and validated for water, sediment and tissue**
- **Method of quantitation - total vs. isomers**
- **Method extends to other pyrethroids – allethrin, flucythrinate, phenothrin, prallethrin, tetramethrin and tralomethrin**
- **GC/MS/MS (TSQ): Very sensitive – at least 2 – 10 times lower than GC/ECD; best tool to obtain lower reporting limit by reducing background interference**

## **FUTURE METHOD DEVELOPMENT**

- Optimize GC/MS/MS analysis by using appropriate internal standards and surrogates
- Complete validation of GC/MS/MS-TSQ
- Complete validation of GC/MS/MS-Ion Trap
- SPME Method Development using MS/MS
- Continue LC/MSD investigation